

BIOLOGY

# *The Inner Life of the Genome*

The way our genes are arrayed and move in the 3-D space of the cell nucleus turns out to profoundly influence how they function, in both health and disease

*By Tom Misteli*

## IN BRIEF

**Chromosomes** are not sprinkled randomly around the inside of the nucleus. They occupy preferred positions.

**This nuclear organization** reflects the functional state of each chromosome and of the genes it carries. The organization can change as a cell's behavior changes and in disease.

**Identifying the locations** that genes occupy within the nucleus—and seeing how these positions change under different conditions—is providing clues to how normal cells function and how some diseases, including cancer, arise.



**Chromosomes** in a dividing cell (*left*) are duplicated and highly compact. At other times, though, they are singletons and more expanded (*below*). Until the recent advent of “chromosome painting” techniques, the expanded chromosomes were difficult to distinguish from one another.



**Tom Misteli** is a senior investigator at the National Cancer Institute in Bethesda, Md. Aided by imaging tools he developed, his laboratory is working to uncover fundamental principles of three-dimensional genome organization in the nucleus of living cells and to apply this knowledge to discovering new strategies for addressing cancer and aging.



**T**EN YEARS AGO PUBLICATION OF THE HUMAN genome sequence gave the world a blueprint for a human being. But just as a list of automobile parts does not tell us how a car engine works, the complete genome sequence—a list of the DNA “letters” in all the chromosomes of the human cell—did not reveal how the genome directs our cells’ day-to-day activities or allows an individual to develop from a fertilized egg into a functioning adult.

To better understand the way the genome as a whole orchestrates the symphony of biological activity called life, I and others in the new field of genome cell biology are examining how chromosomes, and the genes they house, are arranged within the three-dimensional space of the nucleus and how that organization influences their activities.

Aided by new 3-D imaging technology that allows us to peer deeper than ever into the living cell, we have discovered a startlingly vibrant ecosystem. In the nucleus, chromosomes physically interact with neighboring chromosomes, genes on those chromosomes migrate to different nuclear locations depending on what they need to accomplish, and molecules that regulate gene activity congregate in bustling hubs. These new discoveries offer fresh insights into how our genomes maintain our health and how some diseases, including certain cancers, arise; they may also lead to new ways of diagnosing disease.

### EARLY QUESTIONS

THE RECENT PROGRESS grows out of discoveries made in the 1980s. Back then, biologists knew that chromosomes become highly condensed during cell division, taking on the hourglass-shaped structure that most of us picture when we think about the entities that carry our genes from one generation to the next. They also knew that chromosomes have a looser shape when cells are not dividing and are going about their usual business. That relaxed appearance made it hard to discern chromosomes individually even with the very best microscopes, and prevailing opinion held that chromosomes in nondividing cells intermix like spaghetti jumbled up in a bowl.

That view was prevalent in spite of some hints to the contrary. In the early 1900s a German cell scientist named Theodor

Boveri had objected to this “spaghetti model” of chromosome organization. Based on studies he had conducted with a kind of roundworm that infects horses, he argued that, although a chromosome could undergo changes in size and shape throughout the life of a cell, each chromosome occupies a distinct, well-defined area of the nucleus. He christened the regions inhabited by these individual chromosomes as “chromosome territories.” But because chromosomes were difficult to see—and because Boveri’s roundworms

were not a typical experimental system—his concept of chromosome territories long remained marginalized.

Definitive experimental evidence for the chromosome territory idea came only when two other Germans, the brothers Thomas and Christoph Cremer, developed a method for marking and visualizing the genetic material in a small region of the nucleus. In the early 1980s the Cremers showed that when a laser beam hits DNA in a particular area of the nucleus, only a few chromosomes come away branded. If nuclear DNA were as jumbled together as had been previously believed, each laser pulse would have struck many more chromosomes.

A few years later investigators perfected a more targeted and colorful method for tagging and visualizing whole chromosomes. This approach—dubbed chromosome painting—attaches fluorescently labeled tags to sequences of DNA code letters in individual chromosomes. Each chromosome can be tagged with a specific fluorescent marker and its location pinpointed. These studies demonstrated unambiguously that chromosomes exist in the nucleus as distinct entities, occupying a space separate from other chromosomes [*see micrograph on opposite page*].

This finding raised many questions, now being addressed by genome cell biologists. Are chromosomes scattered randomly throughout the nucleus, like attendees at an event with open seating? Or do chromosomes have “assigned seats” within the nucleus? And more important, does their position affect the activity of the genes they harbor?

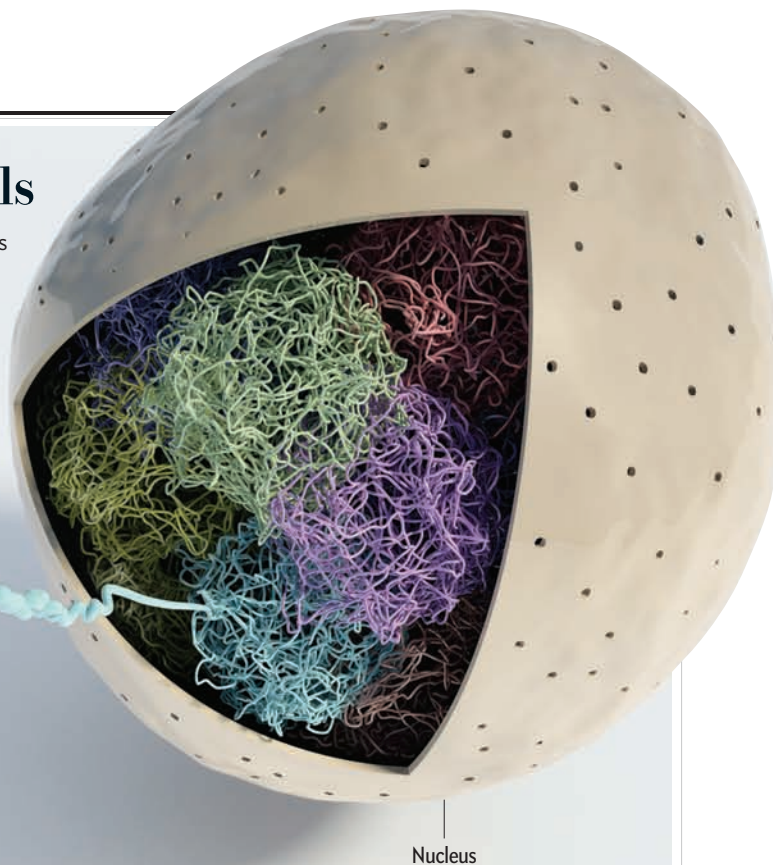
### FAVORED NEIGHBORHOODS

WE NOW KNOW that individual chromosomes tend to occupy preferred locations inside the nucleus. In human white blood cells, for example, chromosome 18 generally hugs the outer wall of



# Organized at Many Levels

Biologists have long known that the DNA in chromosomes folds up in complex ways (*diagram*). They have now also demonstrated that individual chromosomes occupy distinct territories in the nucleus (*micrograph*) and that some chromosomes prefer the nuclear periphery, whereas others like to cluster closer to the core. Moreover, where chromosomes reside, and which chromosomes lie near one another, can strongly influence how cells function.



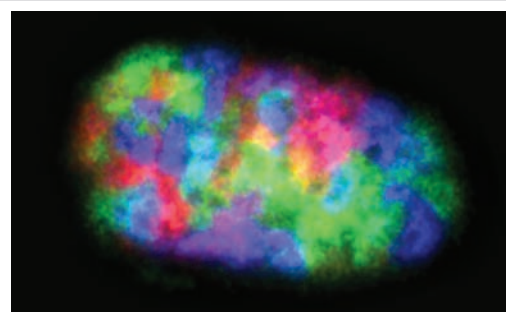
## Architecture of a Chromosome

The DNA in each of our 46 chromosomes is wrapped around spools consisting of histone proteins, and the spooled DNA folds up still more. Collectively, the DNA complexed to proteins is known as chromatin. Laid out end to end, all the nuclear DNA in a whole human body would stretch the distance between the earth and the sun and back again about 100 times.

Chromatin

Histone spool

DNA



## Architecture of a Nucleus

In the past 15 years advanced microscopy has given the lie to a long-standing view that chromosomes sit higgledy-piggledy in the cell nucleus, like cooked spaghetti in a bowl. This image individually colors the chromosomes in the nucleus of a human fibroblast.

the nucleus, whereas chromosome 19 prefers to remain in the center; chromosome 7, meanwhile, tends to hover somewhere in between. The tendency of each chromosome to assume a preferred position either closer to—or farther from—the nuclear edge also creates distinct neighborhoods throughout the nucleus. Consequently, each chromosome has a set of neighbors that is usually consistent from one cell to another within a given type. In studies of mouse white blood cells, for instance, my colleagues and I found that chromosome 12 often clusters with chromosomes 14 and 15.

Chromosome positions are not etched in stone, however. My laboratory discovered that chromosomes are arrayed different-

ly in different cell types, and other researchers have found that these arrangements change during development and in disease. What is more, where a chromosome lives seems to influence whether the genes it carries are turned on or off.

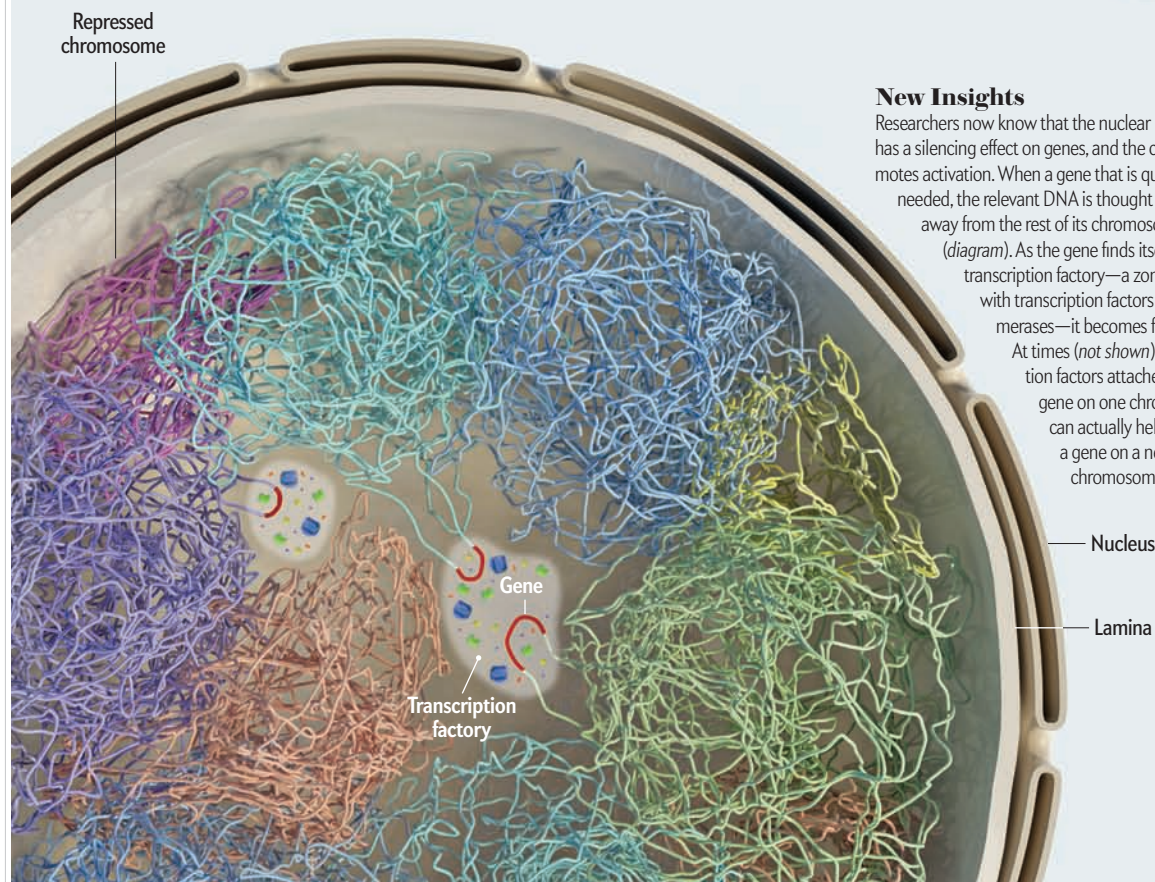
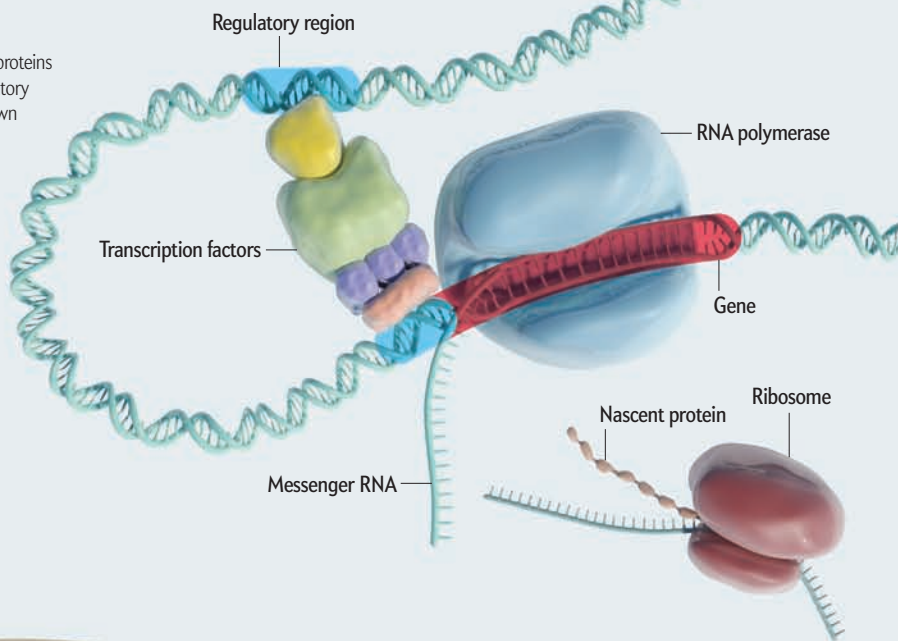
A hint that a gene's location within the nucleus might be important for its activity came from the finding that some genes change their positions when their activity changes. One example comes from studies that tracked a gene called *GFAP*. Star-shaped brain cells called astrocytes typically have one active copy of the gene (the copy used to make a protein specified by the gene) and one silent copy. Takumi Takizawa in my lab discovered that the silent version generally lies toward the periph-

## Fresh Clues to Gene Activation

For many years investigators have had a good understanding of the molecular machines that switch on genes (*top image*), the parts of chromosomes that encode the proteins and RNA molecules produced in cells. But now, thanks to new tools, they also have insight into a higher level of control: that exerted by the architecture of the nucleus (*bottom*).

### Basics of Gene Activation

A gene gets switched on, or read out, after proteins called transcription factors collect on regulatory regions of the gene, enabling enzymes known as RNA polymerases to transcribe the gene's DNA code letters, or nucleotides, into mobile RNA copies. In the case of protein-coding genes, the RNA molecules, known as messenger RNAs, migrate to the cytoplasm, where structures called ribosomes translate them into the specified proteins.



### New Insights

Researchers now know that the nuclear periphery has a silencing effect on genes, and the center promotes activation. When a gene that is quiet is needed, the relevant DNA is thought to loop away from the rest of its chromosome (*diagram*). As the gene finds itself in a transcription factory—a zone buzzing with transcription factors and polymerases—it becomes fully active. At times (*not shown*), transcription factors attached to a gene on one chromosome can actually help activate a gene on a nearby chromosome.



ery of the nucleus, whereas the active copy resides in the nuclear interior. Others have found a similar positioning for genes that encode the defensive antibodies, or immunoglobulins, that white blood cells secrete when provoked by an invader. In white blood cells that have been placed on alert by foreign cells, the region of the chromosome that houses the *IGH* gene, which encodes an immunoglobulin component, tends to move to a more central position in the nucleus. Together, such discoveries have pointed to a simple rule of how the position of a gene affects its function: genes at the periphery of the nucleus are often inactive.

Might something in the outer flanks of the nucleus favor gene silencing? An early sign that the answer is yes was the observation, made in the 1930s, that the nuclear periphery is lined with heterochromatin—chromosomal regions that are highly condensed. If you had supernatural vision and could look inside a chromosome, you would see that it consists of double-helical DNA that is wrapped around spools composed of proteins called histones and that this spooled DNA folds in on itself to form a thick fiber called chromatin [see illustration on page 69]. Chromatin fibers themselves fold up even further, becoming increasingly condensed. Heterochromatin is a special form of chromatin that is coiled particularly tightly, an arrangement that generally prevents gene-reading proteins from accessing the underlying DNA.

Of course, that early observation could not reveal whether the periphery promotes silencing—or whether compacted chromatin is attracted to that area for other reasons. But a set of elegant experiments, conducted by several labs in 2008, favors the first view. When researchers removed active genes from their regular location in the nuclear interior and tethered them to the membrane that surrounds the nucleus, their activity was generally reduced. So the nuclear periphery helps to keep at least some genes quiet.

The nuclear interior, for its part, might also offer something special to chromosomes and genes whose activity is required quickly or often: collections of protein conglomerates known as transcription factories. These “factories” are aggregations of the cellular components required to activate genes, including polymerase enzymes (which transcribe DNA into RNA that is later translated into an encoded protein), as well as transcription factors (proteins that bind to regulatory areas of genes and start the polymerases on their way).

Peter Cook of the University of Oxford first proposed the existence of these factories in 1993, after noting that the number of active genes in the nucleus at any given time is much greater than the number of sites where polymerases are busy reading genes. An obvious way to explain this pattern would be the clustering of multiple genes in hubs of transcriptional activity, where they share polymerases and transcription factors [see box on opposite page]. The idea is not without precedent: hundreds of genes that encode ribosomal RNAs (vital parts of the cell’s protein-producing machinery) are transcribed together in the nucleolus—a nuclear substructure large enough to see under a microscope.

*Chromosomes are arrayed differently in different cell types, and these arrangements change during development. Where a chromosome lives seems to influence whether the genes it harbors are on or off.*

## HEALTH MATTERS

GENOME CELL BIOLOGISTS have not yet learned all the rules governing the activity of genes in different parts of the nucleus. We have shown, however, that where genes reside in nuclear space has relevance to normal development and to health.

A particularly striking instance of how gene organization changes during normal embryonic development has emerged from studies of the embryonic stem cells. These cells are “pluripotent” generalists, possessing the unique ability to differentiate into any one of the 220 or so specialized tissues in the body, such as nerve cells, blood cells or muscle. Unlike fully differentiated cells, these functionally flexible embryonic stem cells lack the large regions of heterochromatin in which genes are silenced.

They also lack proteins called lamins that help to tether inactive DNA to the nuclear periphery. As a result, just about every gene in a stem cell genome is active at a low level.

When embryonic stem cells receive a signal to differentiate into, say, bone cells or neurons, their nuclear architecture changes dramatically. Lamin proteins appear and join together to form a tight, interwoven mat—the nuclear lamina—that sits under the nuclear membrane. This supportive lamina is believed to maintain nuclear shape and to protect the chromosomes from external mechanical pressure. But it also appears to be involved in normal gene regulation. Chromosome segments that have fewer active genes contain a particular structural protein that compresses those regions into heterochromatin—and ties them to the lamin proteins in the outskirts of the nucleus. That sequestration leaves the gene-rich areas closer to the interior and to the gene factories that allow them to be active.

Thus, the appearance of lamins during embryonic development allows cells to shut down genes that are no longer needed, by banishing them to the sidelines.

That this exiling of selected chromosomal regions may be critical for proper gene functioning in differentiated cells is supported by observations of what happens when lamins are abnormal. Mutations in lamins lead to a variety of human disorders, ranging from muscular dystrophies and neurological disorders to premature aging. This collection of so-called laminopathies is unusual in its breadth: in contrast to most conditions—in which any mutation in a given gene leads to the same disease—mutations in lamins cause an unusually broad spectrum of illnesses. Cell biologists are not sure how defective lamins cause these disorders. One possibility is that they weaken the lamina, leaving it unable to shield the nucleus from mechanical forces, with the consequence that much of the genome in vulnerable cells becomes physically damaged, perhaps leading to the cell’s death. Another intriguing idea is that defective lamin proteins may be compromised in their ability to organize the genome, thus placing genes in the wrong places and potentially disrupting their normal functioning.

Studies that have mapped the positions of chromosomes in cells from patients with lamin-based disorders tend to support this last theory: one investigation showed an abnormal relocation of chromosomes 13 and 18—from the periphery to the inte-

rior—in cells harboring a lamin disease mutation. Not yet clear, though, is whether this chromosomal repositioning is a consequence of the disease or a contributing factor.

Chromosomal positioning plays a more clearly central role in some cancers. Malignant cells often contain chromosomal “translocations”—abnormal chromosomes that form when a segment breaks off one chromosome and becomes attached to another [see box on opposite page]. In some cases, such translocations cause cancer because the fusion creates a mutant gene that promotes excessive cell proliferation; in other cases, they are simply bystanders.

As it turns out, which chromosomes combine to form cancer-promoting translocations is influenced by where the chromosomes reside in the nucleus: chromosomes that are found together in the nucleus tend to fuse more frequently. Consider Burkitt's lymphoma. Many patients with this disease have a translocation between the *MYC* gene, located on chromosome 8, and the *IGH* gene, on chromosome 14; in rare cases, *MYC* translocates with a different immunoglobulin gene on chromosome 2, called *IGK*, and even more rarely with the *IGL* gene, on chromosome 22. In 2003 Jeffrey Roix in my lab discovered that the average distance in the nucleus between *MYC* and its three translocation partners corresponds precisely to their translocation frequencies, suggesting a link between gene distance and probability of translocation. The same link has since been found for a number of other cancers.

My lab has also shown that when a chromosome breaks, the damaged ends remain close to home and do not stray far from where they were situated at the time of breakage. This observation explains why chromosomes clustered in the same neighborhood have a greater probability of fusing than distant chromosomes do. It explains, too, why specific translocations are a hallmark of cancers that arise in one tissue but not another: because chromosomes are arranged differently in different tissues. Thus, chromosomes that cluster near one another in, say, kidney cells, would be more likely to be translocation partners in kidney tumors than in cancers of other tissues, such as white blood cells, where they normally lie farther apart.

One of the most exciting developments in the field has been the realization that knowledge of where chromosomes typically reside in the nucleus might present opportunities for cancer detection. Preliminary experiments have demonstrated that the position of genes can help indicate whether a cell is cancerous. In a pilot study of breast cancer, Karen Meaburn in my lab identified several genes whose positions differed in tumor cells as compared with cells from normal breast tissue. These genes turned out to be good markers for breast cancer: they allowed us to pick out cancerous tissue samples with very high accuracy. In malignant cells, some genes change position even before the cells begin behaving badly. We have reason to hope, therefore, that gene-position analyses will one day become a powerful molecular tool for helping physicians to diagnose cancer at very early stages.

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## THE SELF-ORGANIZED NUCLEUS

THE HOLY GRAIL in the field of genome cell biology is the question of what determines where a gene or a chromosome is positioned in the nucleus. How do genes and chromosomes know where to go—and how do they get there as the cells in which they reside differentiate into their specialized states?

One theoretical possibility is that chromosomal sequences get escorted to their proper destinations by specific cellular machinery. Perhaps a DNA-binding protein that recognizes a specific gene sequence attaches to that sequence and then—with the help of a molecular motor protein—drags that part of the chromosome to a particular site in the nucleus. But so far

no one has identified such a system. And it is hard to imagine a signaling system that could communicate a set of geographic coordinates to a piece of DNA, directing a gene to loiter near the nuclear center or to pay a visit to its favorite transcription factory.

Instead I have proposed that nuclear positioning is self-organizing, somewhat like middle school students forming cliques because they are drawn together by mutual interests, not because they were instructed to associate by parents or teachers. In this view, the location of genes and chromosomes inside the nucleus springs from their activity and is not determined by some external organizing machinery. In turn, their location then influences their activity.

How would this self-organization work? Let us follow what happens in a self-organizing nucleus when an individual gene in a differentiated cell is turned on in response to a signal, say, a hormone. Before the signal reaches the cell, the gene is inactive—most likely tucked away in a section of condensed chromatin, perhaps even in a block of heterochromatin hugging

the nuclear periphery. When the signal arrives in the nucleus, molecules known as chromatin remodeling complexes unfold the condensed DNA in and around the gene and make the region more accessible to the transcriptional machinery. In a self-organizing nucleus, this relaxation would allow that stretch of chromatin to loop out from the heterochromatin in the periphery and to flop around, exploring new parts of the nucleus. With any luck, the meandering loop will eventually make contact with a transcription factory.

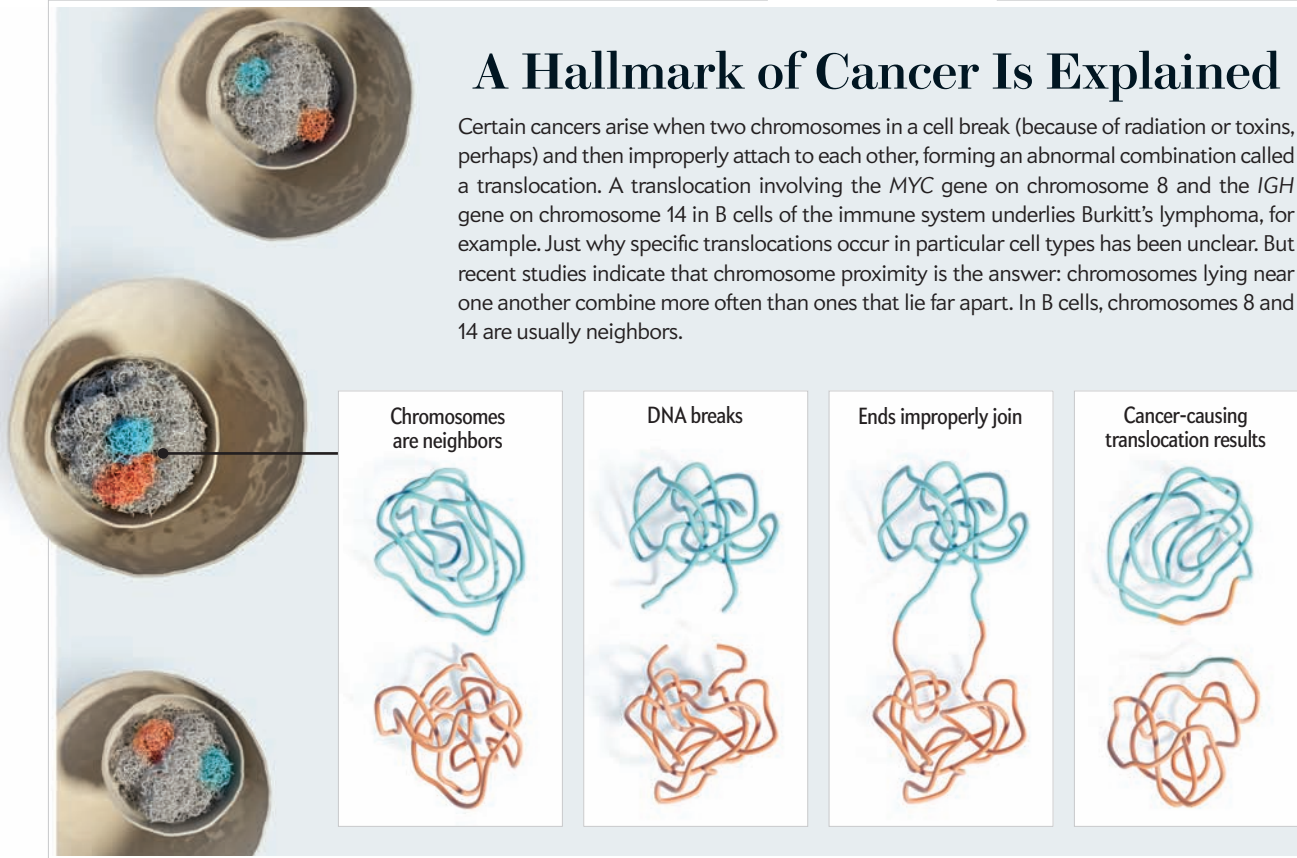
Note that this movement of the gene—from the nuclear outskirts to the center of the action—occurs without the help of a dedicated transport machinery and is entirely driven by the activity of the gene itself. Thus, the position of the gene is self-determined. This model has an intriguing consequence: it suggests that although a gene's nuclear location is not random, how it gets there can be.

The self-organization concept agrees with many results from gene-tracking experiments. Genes can loop out from chromosomes and travel through the nucleus. A few genes even take this transcriptional ticket-to-wander to an extreme. When white blood cells are stimulated by hormones called cytokines, genes that encode immune system proteins known as MHC class II molecules stray far away from the body of the chromosome on

INTERVIEW WITH  
THE AUTHOR  
ScientificAmerican.com/  
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## A Hallmark of Cancer Is Explained

Certain cancers arise when two chromosomes in a cell break (because of radiation or toxins, perhaps) and then improperly attach to each other, forming an abnormal combination called a translocation. A translocation involving the *MYC* gene on chromosome 8 and the *IGH* gene on chromosome 14 in B cells of the immune system underlies Burkitt's lymphoma, for example. Just why specific translocations occur in particular cell types has been unclear. But recent studies indicate that chromosome proximity is the answer: chromosomes lying near one another combine more often than ones that lie far apart. In B cells, chromosomes 8 and 14 are usually neighbors.



which they are located—sometimes stretching halfway across the nucleus.

The same principle may govern the positioning of entire chromosomes. Although most genes are rather subtle in their movement, each makes a small contribution to where its chromosome will wind up in the cell. So if self-organization is the rule, one would expect a chromosome that contains mostly inactive genes will find itself pulled toward the more repressive regions in the nuclear periphery, whereas a chromosome having predominantly active genes will be dragged toward the nuclear interior.

To test this prediction, Mark Groudine and his colleagues at the Fred Hutchinson Cancer Center in Seattle collected blood precursor cells and then triggered their maturation. At different points, cells were harvested, and the activities of several thousand genes were measured. At the same time, the investigators monitored the position of the chromosomes on which these genes were located. The results: the chromosomes that harbored the largest number of genes whose activity changed as the cells matured showed the most movement.

These experiments are a good start, but they are difficult, because it is tedious to simultaneously monitor the position of many genome regions microscopically. A potentially revolutionary method, dubbed Hi-C, may soon solve this problem. This approach, developed by Job Dekker of the University of Massachusetts Medical School, gives an instantaneous snapshot of the three-dimensional architecture of the genome by chemically tying together all the chromosomal regions that

touch one another in the nucleus. Using Hi-C, biologists should soon be able to determine the locations of chromosomes in nuclei from different tissues at different times and under different conditions—and, by comparing these patterns with the sets of active and inactive genes, obtain unprecedented insight into how nuclear organization influences function and how disruptions contribute to disease.

Producing the first draft of the human genome sequence took about 10 years of massive effort. Genome cell biologists, driven to learn more than sequence alone can reveal, are just beginning to uncover the ways genomes behave in their natural habitat of the cell. This task, though exhilarating, is formidable. Given its complexity, it will likely occupy biologists far longer than it took to sequence the human genome in the first place. ■

### MORE TO EXPLORE

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**Cell Biology: Chromosome Territories.** Karen J. Meaburn and Tom Misteli in *Nature*, Vol. 445, pages 379–381; January 25, 2007.

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**Comprehensive Mapping of Long-Range Interactions Reveals Folding Principles of the Human Genome.** Erez Lieberman-Aiden et al. in *Science*, Vol. 326, pages 289–293; October 9, 2009.

**The Nucleus.** Edited by Tom Misteli and David L. Spector. Cold Spring Harbor Laboratory Press, 2010.